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(54) Title: CYCLOSPORINS TO TREAT ALZHEIMER'S DISEASE

(57) Abstract: Non-immunosuppressive, cyclophilin-binding cyclosporins, are useful as neuroprotective agents, e.g. in the prevention or treatment of pathological conditions associated with AB secretion and/or production.

CYCLOSPORINS TO TREAT ALZHEIMER'S DISEASE

The present invention relates to novel uses of cyclosporins, and in particular to new pharmaceutical uses of non-immunosuppressive, cyclophilin binding cyclosporins.

Cyclosporin A (CsA) binds to immunophilin proteins such as cyclophilins (CyP), whereas FK506 and rapamycin, both immunophilin-binding compounds bind to FK506-binding proteins (FKBP). Although immunophilin-binding is required, it is not sufficient for the immunosuppressive activity of these drugs. Biological effects are observed upon interaction of the drug/immunophilin complexes with a third effector protein. For example, the CyP-CsA and FKBP-FK506 complexes inhibit the serine/threonine phosphatase activity of calcineurin, thereby blocking the production of cytokines such as interleukin-2.4 On the other hand, the FKBP-rapamycin complex inhibits a kinase called FRAP (also known as RAFT or mTOR) 5 that is involved in interleukin-2 receptor-mediated T cell proliferation.

A new class of compounds named sanglifehrins, have been isolated from *Streptomyces sp.* A92-308110. Among the 20 different sanglifehrins isolated so far, sanglifehrin A (SFA) is the most abundant compound and displays potent immunosuppressive activity. SFA represents a novel type of immunosuppressant whose mode of action is different from that of all other known immunophilin-binding compounds, namely CsA, FK506, and rapamycin. SfA has been shown to bind directly to the immunophilin protein, cyclophilin D (CyD), at a distinct site from another immunophilin, cyclophilin A (CpA), at the mitochondrial transition pore (MTP) complex and inhibit MTP opening.

Non-immunosuppressive, cyclophilin binding cyclosporins and their use in the treatment and prevention of AIDS and AIDS-related disorders are described in European Patent no. 484281, which includes a general description of the cyclosporin class of compounds, their nomenclature and mode of action. The disclosure of EP 0,484,281 B, in particular the general description referred to above and other parts of the description referred to hereinafter, is included by reference in the teaching of the present application.

Surprisingly, it has now been found that cyclosporins which bind to cyclophilin, but are not immunosuppressive, are useful as neuroprotective agents to treat pathological conditions associated with Aβ production and/or secretion, including but not limited to Alzheimer's Disease ('AD"). AD is characterized by the extracellular accumulation of amyloid plaques in the brain composed primarily of the Aβ 40 or 42 amino acid peptides. The extracellular accumulation of these peptides is a hallmark pathology of the disease (Selkoe 1999). The Aβ peptide is generated by the endoproteolytic cleavage of the amyloid precursor protein (APP), a ubiquitously expressed type I transmembrane protein (Selkoe 1999; Sisodia 2000). The two enzymes that cleave APP in the amylogenic pathway are called the β- and γ-secretases, which cleave APP from the N- and C-termini, respectively. In this pathway, the β-secretase (BACE1) is the first enzyme to cleave APP, producing a secreted sAPPβ fragment and a membrane associated C-terminal fragment (CTF, C99) (Vassar, Bennett et al. 1999). The C99 fragment is the substrate for the γ-secretase complex (GACE) which cleaves C99 to produce Aβ and AICD (APP IntraCellular Domain). AICD binds a complex with Tip60 and Fe65 which derepresses KAI1 (a tetraspanin cell surface molecule), a gene in the NFκ-B pathway (Baek, Ohgi et al. 2002). The GACE complex is made up of four primary components, presenilin 1 (PS1), nicastrin (NCSTN), Aph1, and Pen2 (Edbauer, Winkler et al. 2003; Kimberly, LaVoie et al. 2003). The PS1 functional homologue, presenilin 2 (PS2) contributes to ~20% of the Aβ produced in a cell (Kimberly, Xia et al. 2000). Although these four components are necessary and sufficient to reconstitute GACE activity, there is evidence of non-GACE mediated cleavage of APP resulting in Aβ production (Tesco, Koh et al. 2003), (Nunan, Shearman et al. 2001).

DETAILED DESCRIPTION OF THE INVENTION

The elucidation of novel genes involved in the APP pathway is a critical step in determining complete etiology of the disease as well as developing a better understanding of the complex mechanism driving Aβ production. The determination of new genes and pathways regulating Aβ aids in developing novel therapeutic strategies to treat the progression of the disease. Several genetic links and chromosomal regions have been specifically associated with Late Onset Alzheimer's Disease (LOAD) (>65 years of onset) (Ertekin-Taner, Graff-Radford et al. 2000),(Bertram, Blacker et al. 2000), (Scott, Hauser et al. 2003). A subset of LOAD associated genes, as well as novel genes involved in APP processing are described in copending USSN __, which includes a description of large-scale

functional screen to test cDNA clones for their ability to modulate Aβ production in CHO K1 cells. The disclosure of USSN __, in particular the identification of genes which modify AB secretion and other parts of the description referred to hereinafter, are included by reference in the teaching of the present application.

The discovery of genes found in the immunophilin pathway which serve as critical regulators of Aβ production in cells provides suitable drug targets in Alzheimer's disease . Some of the cDNAs discovered in the functional screen which are implicated in the immunophilin pathway include Map kinase 4, Calmodulin, acid ceramidase, and TOB3, a AAA-ATPase.

Mitogen-activated protein kinases (MAPKs), also known as extracellular signal-regulated kinases (ERKs), are a family of serine/threonine protein kinases that phosphorylate and positively or negatively regulate target substrates initiating signaling cascade events. ERKs transduce signals from the cell membrane to the nucleus in response to a wide range of stimuli and play an important role in modulating gene expression, mitosis, proliferation, motility, metabolism, and apoptosis (Wada and Penninger 2004). Inhibitors of MEK and ERK activity are shown to inhibit APP catabolism. In addition, MAPK can activate JNK and trigger apoptosis, a process known to increase Aβ production (Tesco, Koh et al. 2003). Although not wishing to be bound by theory, the MAPK4 cDNA could be acting through APP catabolism or activation of apoptosis.

As well, the APP intracellular domain (AICD) fragment interacts with cJun N-terminal kinase (JNK) linking the MAP kinase pathway to APP processing (Scheinfeld, Roncarati et al. 2002). MAPK4 is an ERK that activates JNK and therefore the overexpression of MAPK4 could lead to hyperactivation of JNK which could increase JNK-AICD interactions leading to increased Aβ production. It is also possible the activation of JNK could induce apoptosis. Alternatively, MAPK4 could alter the phosphorylation state of APP which has been shown to affect its preferential processing by the β- and α-secretase by affecting the trafficking of APP and disrupting PS1/β-catenin interactions (Hung and Selkoe 1994), (Walter, Capell et al. 1997). Additionally, the phosphorylation state of the cells has also been shown to be critical for PS1 activity (Seeger, Nordstedt et al. 1997). JNK activation and protein phosphorylation are also several other pathways MAPK4 could be acting to increase Aβ production.

Another gene identified in the screen is Calmodulin. Calmodulin is a loop-helix-loop Ca^{2+}-binding protein that transduces Ca^{2+} signals by interacting with specific target proteins

including CaMKII, CaMKIV, calcineurin, spectrin A2, p21, and neuronal nitric oxide synthase (Means 1981). When Ca^{2+} binds Calmodulin it undergoes a conformational change that allows it to bind to target proteins and to stimulate or inhibit their activities. Calmodulin has been shown to regulate Aβ production in cell-free preparations and intact cells using the Calmodulin antagonists W-7 and trifluoperazine. These known inhibitors of Calmodulin activity can also inhibit Aβ production (Desdouits, Buxbaum et al. 1996). Thus, intracellular Ca^{2+} concentrations and/or calmodulin target proteins can affect APP processing. This compound data validates the fact that an increase in Aβ production is observed when Calmodulin is overexpressed.

Ca^{2+} dysregulation, particularly a drop in cytoplasmic Ca^{2+} levels, is concomitant with an increase in Aβ production associated with PS1 FAD mutations (Yoo, Cheng et al. 2000). PS1 FAD mutations can significantly attenuate capacitative calcium entry (CCE) and store depletion-activated currents suggesting reduced CCE can increase Aβ generation (Yoo, Cheng et al. 2000). The overexpression of the calmodulin gene found in the screen could also cause a drop in intracellular calcium levels, and could prevent adequate CCE. It is likely the decrease in intracellular Ca^{2+} could increase PS1 activity, either directly or indirectly, resulting in more Aβ secretion from cells. Data described in detail below suggests that SfA potently inhibits Aβ40 & Aβ42 production and inhibits C99 and Notch cleavage suggesting gamma secretase activity is linked to Ca^{2+} homeostasis through the immunophilin protein CyD.

Another gene, human acid ceramidase catalyzes the hydrolysis of ceramide to sphingosine and fatty acid (Ferlinz, Kopal et al. 2001). Ceramide serves as the precursor for most sphingolipids and is a signaling molecule that induces apoptosis in a number of different cell types, typically through caspase-3 activation. Accumulating ceramide levels are associated with Alzheimer's disease and are thought to be part of the oxidative neurotoxic pathway in the aging AD brain. Overexpression of ceramide increases Aβ production linking ceramidase cleavage to Aβ. Since ceramide seems to act though apoptosis it is likely the acid ceramidase is increasing apoptosis as well.

The ceramide status of the cell has been shown to regulate both BACE stability and Aβ biogenesis (Puglielli, Ellis et al. 2003). Previous studies demonstrate that high levels of ceramide can increase Aβ secretion in an apoptosis independent fashion (Puglielli, Ellis et al. 2003). Ceramidase overexpression would decrease ceramide levels and increase sphingosine and free fatty acid levels (FFA) possibly altering membrane fluidity, a process

known to alter tau phosphorylation and Aβ polymerization (Wilson and Binder 1997). Although the mechanism is unclear, it could be that ceramidase can affect the FFA levels leading to altered APP processing and more Aβ production and/or secretion.

TOB3 is a AAA-ATPase that performs chaperone-like functions that assist in the assembly, operation, or disassembly of protein complexes, including protein secretion (Strausberg, Feingold et al. 2002). This class of proteins helps to maintain the integrity of the endoplasmic reticulum (ER) as proteins are being constitutively processed. In the absence of AAA-ATPase activity there is excessive accumulation of misfolded proteins causing ER expansion and cell death (Kobayashi, Tanaka et al. 2002). The most likely explanation of TOB3 overexpression increasing Aβ secretion is linked to its ability to effect protein folding and trafficking in the ER. If this process is stimulated, as with TOB3 overexpression, it is possible APP processing would be increased.

Our screening data has shown that TOB3 overexpression alters APP processing leading to more Aβ production and fewer C99 & C83 C-terminal fragments. TOB3 expression also decreases APP and sAPPα levels in N2A cells to a greater extent than HEK 293 cells (data not shown). This suggests that TOB3 affects one or more components of the APP processing machinery resulting in increased APP and C99 cleavage. Since TOB3 is involved in the transport and processing of proteins, the exact mechanism and specificity of TOB3 on APP processing is currently being determined.

Carboxypeptidase Z (CPZ), another cDNA identified from the functional screen, is a member of the metallocarboxypeptidase gene family along with CPE and CPD which are thought to function in the intracellular processing of bioactive peptides and proteins prior to their secretion. CPZ is a unique carboxypeptidase because it contains a functional N-terminal cysteine-rich frizzled domain that binds to Wnt and wingless proteins which are a critical component of Wnt signal transduction (Moeller, Swindell et al. 2003).

Another interesting cDNA is cyclophilin D (CyD, also known as CyF. Reference to nomenclature can be found in Current Medicinal Chemistry, 2003, 10, 1485-1506 1485 Cyclophilin D as a Drug Target, Waldmeier, et al). CyD was found to be involved in Aβ production. Cyclophilin proteins, such as CyD, are peptidyl prolyl isomerases involved in protein trafficking and maturation. Cyclophilins are primarily localized to the cytoplasm except CyD which is exclusively localized in the mitochondrial matrix (Waldmeier, Zimmermann et al. 2003). CyD when overexpressed, could stabilize endogenous PS1 N-terminal fragments (NTFs). A common lesion of the AD brain is the presence of intracellular

neurofibrillary tangles made up of abnormally phosphorylated tau, a microtubule-associated protein (Johnson and Bailey 2002). When overexpressed with APP, CyD could increase caspase-3 activity, indicating a possible mechanism through which Aβ secretion is modulated. These results suggest that when overexpressed, CyD is an important factor required for the cleavage of APP and C99 through the γ-secretase pathway.

In one aspect, CyD is an integral constituent of the mitochondrial permeability transition pore thought to bind the adenine nucleotide translocator and regulate pore opening (Waldmeier, Zimmermann et al. 2003). Permeablization of mitochondrial membranes is a consequence of cellular stress that leads to dissipation of the mitochondrial membrane potential, release of apoptogenic proteins, and culminates in apoptotic cell death (Waldmeier 2002).

Mitochondrial failure has been suggested to play a significant role in the development of AD neuropathology in Down's syndrome patients, by promoting aberrant β-APP processing and intracellular accumulation of Aβ (Busciglio 2002). Increases in intracellular calcium levels has also been shown to elicit accumulation of intracellular Aβ (LaFerla 2002). Surprisingly, full length APP does not only traffic along the secretory pathway, but it is also targeted to mitochondria of cultured cortical neuronal cells in the brain of a transgenic mouse model for AD. Incomplete translocation and progressive accumulation of β-APP on mitochondrial membranes can lead to mitochondrial dysfunction and can also play a role in the pathogenesis of AD (Anandatheerthavarada, Biswas et al. 2003). It is known that overexpression of mitochondrial proteins can lead to the formation of insoluble aggregates within the matrix (e.g. uncoupling protein-3, UCP3). These aggregates can both derange the normal function of mitochondria and increase the passive permeability of the inner membrane. CyD may play an important role in the mitochondria dealing with excess APP or C-terminal fragment processing.

CyD is known target of the immunosuppressant drugs such as cyclosporin. These compounds block mitochondrial permeability transition (MPT) and prevent apoptosis (Samantha J. Clarke 2002; Waldmeier 2002). FK506 compounds are also known to bind immunophilin proteins but do not bind CyD (Uchino 2003).

A cyclosporin is considered as binding to cyclophilin if it binds to human recombinant cyclophilin at least one fifth as well as does Ciclosporin (also referred to as cyclosporin A) in

the competitive ELISA test described by Quesniaux in Eur. J. Immunol. 1987, 17, 1359-1365. In this test, the cyclosporin to be tested is added during the incubation of cyclophilin with coated BSA-Ciclosporin and the concentration required to give a 50% inhibition of the control reaction without competitor is calculated (IC_{50}). The results are expressed as the Binding Ratio (BR), which is the log to the base 10 of the ratio of the IC_{50} of the test compound and the IC_{50} in a like test using Ciclosporin in place of the test cyclosporin. Thus a BR of 1.0 indicates that the test compound binds cyclophilin one factor of ten less well than does Ciclosporin, and a negative value indicates binding stronger than that of Ciclosporin.

The cyclosporins active as neuroprotective agents have a BR lower than 0.7, (since $\log_{10} 5 =$ 0.7 approx), preferably equal to or lower than zero.

A cyclosporin is considered to be non-immunosuppressive when it has an activity in the Mixed Lymphocyte Reaction (MLR) of no more than 5%, preferably no more than 2%, that of Ciclosporin. The Mixed Lymphocyte Reaction is described by T. Meo in "Immunological Methods", L. Lefkovits and B. Peris, Eds., Academic Press, N.Y. pp. 227-239 (1979). Spleen cells (0.5×10^6) from Balb/c mice (female, 8-10 weeks) are co-incubated for 5 days with 0.5×10^6 irradiated (2000 rads) or mitomycin C treated spleen cells from CBA mice (female, 8-10 weeks). The irradiated allogeneic cells induce a proliferative response in the Balb c spleen cells which can be measured by labelled precursor incorporation into the DNA. Since the stimulator cells are irradiated (or mitomycin C treated) they do not respond to the Balb/c cells with proliferation but do retain their antigenicity. The IC_{50} found for the test compound in the MLR is compared with that found for Ciclosporin in a parallel experiment.

It has been found that compounds which are judged as non-immunosuppresive in the MLR above are often inactive in an IL-2 Reporter Gene Assay, and thus an IL-2 Reporter Gene Assay may be used, e.g. as a primary screen, for selection of non-immunosuppressive, cyclophilin-binding cyclosporin compounds for use in the invention.

The non-immunosuppressive, cyclophilin-binding cyclosporin compounds which are active as agents to treat pathological conditions associated with Aβ secretion, e.g. as inhibitors of extracellular accumulation of amyloid plaques in AD are hereinafter referred to as Active Compounds.

The active compounds are therefore useful in the treatment of any clinical condition involving Aβ peptide secretion, expression of endogenous PS1 N-terminal fragments (NTFs) or increased gamma-secretase activity. Further the active compounds are useful in the treatment of conditions involved in apoptosis that increase Aβ secretion. Aβ peptide formation and subsequent aggregation are not only a hallmark of AD, but are also an integral part of other neurological disease such as Parkinson's, Huntington's, and other systemic amyloidosis (Selkoe 1989; Price, Borchelt et al. 1993; Citron, Vigo-Pelfrey et al. 1994). It is clear from these results that apoptosis and Aβ production are integrally linked.

The active compounds also have utility to modulate "peripheral Aβ modifiers" not expressed in the brain. Peripheral amyloidosis can result in such phenotypes as cardiac and dermatological amyloidosis (Yamaguchi, Yamazaki et al. 1992), (Selkoe 1989). If a key regulator of peripheral Aβ is found, it is also possible to derive novel therapeutics such to such targets which prevent the need to penetrate the blood brain barrier. A drop in peripheral Aβ levels has been shown to decrease levels of Aβ in the transgenic mouse brain, resulting in less plaque formation (Bohrmann, Tjernberg et al. 1999; DeMattos, Bales et al. 2002).

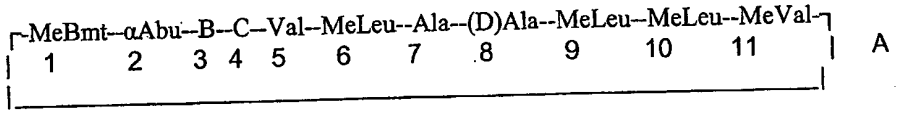
It is found that many of the Active Compounds have structures differing from that of Ciclosporin specifically at the 4 and/or 5 positions. Other positions at which the structures of the Active Compounds may differ from that of Ciclosporin are positions 6 and 7.

One group of Active Compounds are cyclosporins in which the MeLeu group at position 4 is replaced by a different N-methylated amino acid for example γ-hydroxy-MeLeu, Melle, MeVal, MeThr, MeAla, Me Tyr or MeTyr(O-PO(OH)$_2$), or Pro. In addition to Melle and MeThr, the allo-forms Mealle and MeaThr may also be used. In the allo-form, the stereochemistry at the β-position has the opposite configuration to that of the natural amino acid, so that the normal form and the allo-form constitute a pair of diastereoisomers.

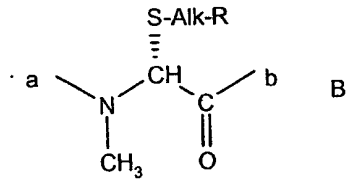
A further group of Active Compounds is that in which Val at the 5-position is replaced by an N-alkyl-, preferably N-methyl-, amino acid. Preferably the amino acid which is N-alkylated is Val or Leu. Preferably the hydrogen of the imino group of $[Val]^5$ is replaced by a non-branched C_{1-6}alkyl group, preferably methyl, ethyl or n-propyl, particularly methyl. The latter preferred group of Active Compounds are all novel.

Additionally or alternatively, certain Active Compounds may differ from Ciclosporin at the 1, 2, 3, and/or 6 positions.

A particular class of Active Compounds for use in the present invention are Ciclosporin derivatives of formula A



wherein B is an amino acid residue of formula B



wherein a denotes the bond to the αAbu residue in position 2;

b denotes the bond to the the residue C in the 4 position;

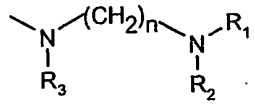
Alk represents straight or branched chain alkylene containing from 2 to 6 carbon atoms or cycloalkylene containing from 3 to 6 carbon atoms, and

R represents

a carboxy or alkyloxycarbonyl radical;

a radical $-NR_1R_2$ in which R_1 and R_2 are the same or different and represent hydrogen, alkyl, C_{2-4} alkenyl, C_{3-6}cycloalkyl, phenyl (optionally substituted by halogen, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino) or a benzyl or saturated or unsaturated heterocyclyl radical containing 5 or 6 ring atoms and 1 to 3 heteroatoms, or in which $R_1$and R_2 form together with the nitrogen atom to which they are attached a saturated or unsaturated heterocycle containing 4 to 6 ring atoms and optionally containing a further heteroatom selected from nitrogen, oxygen or sulphur and optionally substituted by alkyl, phenyl or benzyl;

a radical of formula

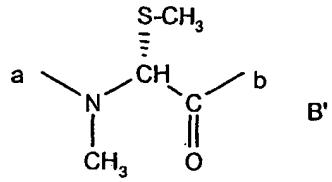


wherein R_1 and R_2 are as defined above, R_3 represents hydrogen or an alkyl radical and n is a whole number from 2 to 4,

and wherein alkyl denotes straight or branched chain alkyl containing from 1 to 4 carbon atoms;

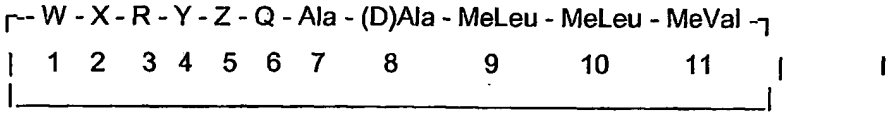
C is MeLeu or or 4-hydroxy-MeLeu; and the pharmaceutically acceptable salts thereof.

This class of Ciclosporin derivatives is further described in published International patent applications Nos. WO 98/28328, WO 98/28329 and WO 9828330. A particularly preferred compound of this class is the compound of formula A in which B is the amino acid residue B'



and C is the amino acid residue 4-hydroxy-MeLeu.

A particularly preferred group of Active Compounds is constituted by the compounds of Formula I:



in which W is MeBmt, dihydro-MeBmt or 8'-hydroxy-MeBmt;

X is αAbu, Val, Thr, Nva or O-methyl threonine (MeOThr);

R is Sar or (D)-MeAla;

Y is MeLeu, γ-hydroxy-MeLeu, MeIle, MeVal, MeThr, MeAla, Me Tyr, MeTyr(O-PO(OH)$_2$), MeAlle or MeaThr, or Pro;

Z is Val, Leu, N-Alk-Val or N-Alk-Leu

wherein Alk represents Me or Me substituted by

vinyl optionally substituted by

phenyl, or an N S or O heteroaryl containing 6 ring members, or

phenyl optionally substituted by

halogen; and

Q is MeLeu, γ-hydroxy-MeLeu or MeAla and the pharmaceutically acceptable salts thereof.

The groups W,X,Y,Z and Q have, independently, the following preferred significances:

W is preferably W' where W' is MeBmt or dihydro-MeBmt;

X is preferably X' where X' is αAbu or Nva, more preferably X" where X" is αAbu;

Y is preferably Y' where Y' is γ-hydroxy-MeLeu, MeVal, MeThr, MeAla or MeTyr(O-PO(OH)$_2$);

Z is preferably Z' where Z' is Val or MeVal; and

Q is preferably Q' where Q' is MeLeu;

One especially preferred group of Active Compounds are the compounds of Formula I in which W is W', X is X', Y is Y', Z is Z' and Q is Q'.

Particularly preferred Active Compounds of Formula I are:

a) [dihydro-MeBmt]^1-[γ-hydroxy-MeLeu]^4-Ciclosporin,

b) [MeVal]^4 -Ciclosporin,

c) [MeIle]^4 -Ciclosporin,

d) [MeThr]^4 -Ciclosporin,

e) [γ-hydroxy-MeLeu]^4 -Ciclosporin,

f) [Nva]^2-[γ-hydroxy-MeLeu]^4-Ciclosporin,

g) [γ-hydroxy-MeLeu]^4-[γ-hydroxy-MeLeu]^6 -Ciclosporin,

h) [MeVal]^5-Ciclosporin,

i) [MeOThr]^2-[(D)MeAla]^3-[MeVal]^5-Ciclosporin,

j) [8'-hydroxy-MeBmt]^1 -Ciclosporin,

k) [MeAla]^6-Ciclosporin,

l) [DMeAla]^3-[MeTyr(OPO(OH)$_2$)]^4-Ciclosporin,

m) [N-Benzyl-Val]^5-Ciclosporin,

n) [N-5-Fluoro-Benzyl-Val]^5-Ciclosporin,

o) [N-Allyl-Val]^5-Ciclosporin,

p) [N-3-Phenyl-Allyl-Val]^5-Ciclosporin,

q) [Pro]^4-Ciclosporin

Especially preferred Active Compounds are [MeIle]^4-Ciclosporin and [γ-hydroxy-MeLeu]^4-Ciclosporin, most especially [MeIle]^4 –Ciclosporin.

In addition to the compounds of Formula I, preferred Active Compounds include, for example

r) [γ-hydroxy-MeLeu]^9-Ciclosporin.

The Active Compounds may be obtained by methods including:
1) Fermentation
2) Biotransformation
3) Derivatisation
4) Partial Synthesis
5) Total Synthesis.
These methods are described generally and more specifically in Examples 1 to 10 of EP 0484281 B and US Pat. No. 5767069. This general description and the teaching of these Examples are incorporated by reference in the present application. Example 11 of EP 0484281 B describes measurement of the immunosuppressive and cyclophilin-binding activities of representative Active Compounds relative to Ciclosporin, and the teaching of this example is also included within the disclosure of the present application.

Thus the invention provides use of a nonimmunosuppresive, cyclophilin-binding cyclosporin in the manufacture of a medicament for treating or preventing pathological conditions associated with AB secretion such as Alzheimer's Disease, Parkinson's Disease, tauopathies, prion diseases, frontotemporal dementia, striatonigral degeneration, Lewy body dementia, Huntington's disease, Pick's disease, amyloidosis, and other neurodegenerative disorders associated with excess AB production.

The invention further provides a method for the treatment or the prevention of preventing pathological conditions associated with AB secretion such as Alzheimer's Disease, Parkinson's Disease, tauopathies, prion diseases, frontotemporal dementia, striatonigral degeneration, Lewy body dementia, Huntington's disease, Pick's disease, amyloidosis, and other neurodegenerative disorders, comprising administering to said patient an effective amount of an Active Compound of the invention.

The Active Compound may be administered by any conventional route, in particular enterally, e.g. orally, for example in the form of solutions for drinking, tablets or capsules or parenterally, for example in the form of injectible solutions or suspensions. By the intravenous route an indicated daily dosage may be from 1 to 20 mg/kg, preferably from 3 to 10 mg/kg, and by the oral route from 1 to 50 mg/kg, preferably from 10 to 30 mg/kg.

The toxicity of the Active Compounds is believed to be less to that of Ciclosporin. As the Active Compounds are not immunosuppressive, certain side effects of Ciclosporin related to immunosuppression are avoided. Other side effects associated with Ciclosporin, particularly nephrotoxicity and central nervous system toxicity in long term use, are conveniently less than with Ciclosporin.

Preferred galenic formulations for the Active Compounds include those based on microemulsions as described in British Patent Application 2 222 770A, which include topical as well as oral forms; also oral and injectable forms obtained from solid solutions comprising a fatty acid saccharide monoester, e.g. saccharose monolaurate, as described in British Patent Application 2 209 671A. Suitable unit dosage forms for oral administration comprise e.g. from 25 to 200mg Active Compound per dosage.

Formulation Examples A, B, C and D of EP 0484281 B are incorporated herein by reference:

The individual components of these formulations, as well as the methods for their preparation, are fully described in British Patent Application 2 222 770, the contents of which are incorporated herein by reference.

The usefulness of the active compounds as neuroprotective agents may be demonstrated in in vivo or in vitro tests, e.g:

The active compounds of the invention can be provided alone, or in combination, or in sequential combination with other agents. For example, the active compounds of the invention can be administered in combination with anti-inflammatory agents such as but not limited to corticosteroids following stroke or spinal cord injury as a means for blocking further neuronal damage and inhibition of axonal regeneration, Neurotrophic factors such as NGF, BDNF or other drugs for neurodegenerative diseases such as Exelon™ or Levodopa. As

used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The structure of the active ingredients identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active ingredients and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both *in vitro* and *in vivo*.

For the indications mentioned above, the appropriate dosage will, of course, vary depending upon, for example, the particular molecule of the invention to be employed, the mode of administration and the nature and severity of the condition being treated.

EXAMPLES

The following methods are performed to conduct the examples disclosed below:

Transfections. CHO K1 cells (ATCC, Manassas, VA) are plated with DMEM, 10% Fetal Bovine Serum, 5% Penn/Strep, and 22mg of L-Proline (Sigma Chemical, St. Louis, MO). The plates are incubated overnight at 37°C in water jacked CO_2 cell culture chambers. The cDNA of interest is co-transfected with full length APP in a 1:15 ratio (cDNA:APPwt(695)) using Qiagen® SuperFect reagent is used according to the manufacturer's directions. In 6-well dishes, 5x 10^5 cells are plated with DMEM, 10% Fetal Bovine Serum, 5% Penn/Strep (Sigma Chemical, St. Louis, MO) and grown up for 24 hours. The SuperFect mix is made up with 100µl of serum free medium (DMEM), 3µg of total DNA, and 20µl of SuperFect. The media is removed from the cells and 1mL of fresh media is added. The entire SuperFect mix is added to the media and incubated at 37°C for 2 hours. The mixture is then removed and the cells are washed once with 3mL of PBS. Fresh media is added back to the cells and they are incubated for 24 or 48 hours.

Immunocytochemistry – CHO cells are transfected in OPTIMEM (serum-free media) with appropriate constructs using Lipofectamine as described by manufactures (Life Technologies). The CHO cells are immunostained and immunoreactivity is observed as described in Dev et al 1999, *Neuropharmacol.,* (1999) 38, 635-644. Flag-protein expression is detected using anti-Flag M2 monoclonal mouse antibody (Sigma), the secondary antibody is Texas Red-X goat anti-rabbit IgG (Molecular Probes).

Flow Cytometry analysis of caspase-3. HEK cells are transiently transfected with CyD or CPZ along with APPwt for 24 hours. Cells are fixed, permeabilized, and stained using the Caspase-3 apoptosis kit from BD Biosciences (#550914). FITC stained cells are gated from $\geq 10^1$ and measured as cells positive for active caspase-3. Forward and side light scatter are also examined to check the health of the cell population.

Compound treatment. HEK 293 cells stably expressing the APPswe mutant are treated for 24 hours with cyclosporin A, Sangliferhin A, FK506 (as described in Sedrani, et al., J. Am. Chem. Soc. 125, pp 3849-3859 (2003) incorporated by reference in its entirety) and N-methyl-4-valine-cyclosporin concentrations. Cell viability is measured using the CellTiter-Glo Kit from Promega (#G7573). Aβ40 & 42 levels are measured in the cell supernatants using an in house two-sandwich ELISA (the in-house ELISA is the same format as described except antibodies developed at Novartis are used instead of the Biosource antibodies). A cell line stably expressing a 5xGal4 response element-luciferase reporter and a C99-Gal4-VP16 or a Notch-Gal4-VP16 construct is used to measure C99 or Notch cleavage (as disclosed in Maltese, Wilson et al. 2001). When cleaved by γ-secretase, Gal4-VP16 is released and binds to the reporter increasing luciferase activity. The stable cells are treated with the compounds and the IC_{50} values are determined.

Aβ ELISA. A commercially available mouse monoclonal antibody directed to the NH_2 terminus of the Aβ peptide is used as the capture antibody in pre-coated 96 well plates (Biosource Cat#KBH3481/PPO81 for Aβ40 and Cat#KBH3441/PPO81 for Aβ42). Polyclonal detection antibodies are obtained from Biosource (anti-hAβ40 Cat#44-348 and anti-hAβ42 Cat#44-344) and diluted 1/220 in 15mM sodium azide. The secondary antibody (Biosource Cat#KBH3481 for Aβ40 and Cat#KBH3441 for Aβ42) is a horseradish peroxidase labeled anti-rabbit IgG. The secondary antibody is diluted 1/100 in 3.3mM thymol. The antibody-

coated plates are washed 4x in PBS-TE (1mM EDTA and 0.05% Tween 20, wash buffer) on a microplate washer (Bioteck Instruments, Inc, Winooski, VT) prior to use. 100 µl of the transfected cell's conditioned media is removed and diluted 1:2 in sample diluent containing 1mM AEBSF (Biosource, Camarillo, CA). 100 µl of this mixture is added to the washed, antibody coated 96 well plate, covered with tape, and incubated at 4°C overnight. The samples are removed and the plates are washed 4x with wash buffer. Detection antibody solution is added at 100µl/well and the plates are incubated at room temperature for 2 hours while shaking. The plates are washed again 4x with wash buffer and the secondary antibody solution is added at 100 µl/well and incubated for 2 hours while shaking. The plates are washed 5x in wash buffer and patted dry on a paper towel. 100 µl of stabilized chromogen (tetramethylbenzidine) is added to each well and the plate is incubated for 30 minutes in the dark. 100 µl of stop solution (1N H_2S) is added to the plates to stop the reaction. The plates are read on a microplate reader at 450 nM (Molecular Devices) within one hour.

Antibodies and Western blot analysis. The cDNA for APP wild type and the APP Swedish mutant are inserted into the pCI plasmid expression vector downstream of a cytomegalovirus promoter as previously described (Promega, Madison, WI) (Bodendorf, U., Fischer, F., Bodian, D., Multhaup, G., Paganetti, P. 2001 *J. Biol. Chem.* **276**:12019 12023).
PS1 NTF antibody recognizes the N-terminus of PS1(Thinakaran, Borchelt et al. 1996). Cultured HEK 293 cells are extracted at 24hr post-transfection in RIPA buffer (10 mM Tris, pH 7.5, 150 mM sodium chloride, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodium-deoxycholate, 1% SDS) containing protease inhibitors (Complete® Roche Molecular Biochemicals) and centrifuged at 4 °C for 10 min at 10,000 x g. The supernatants are collected and the pellets discarded. Subsequently, the cell extracts were resolved by SDS polyacrylamide gel electrophoresis, transferred to PVDF Immobilon-P® membranes (Millipore), and probed with primary antibodies as indicated. Immunological detection is carried out with the ECL detection system (Amersham Pharmacia Biotech, Piscataway, NJ) as previously described (Manni, M., et al., 1998. *FEBS.* **427**:367-370).

Immunocytochemistry – CHO cells are transfected in OPTIMEM (serum-free media) with appropriate constructs using Lipofectamine as described by the manufacturer (Life Technologies). The CHO cells are immunostained and immunoreactivity is observed as described previously (Dev et al 1999, *Neuropharmacol.,* (1999) 38, 635-644). Flag-protein

expression is detected using anti-Flag M2 monoclonal mouse antibody (Sigma), the secondary antibody is Texas Red-X goat anti-rabbit IgG (Molecular Probes).

CPZ Constructs- Wild-type human Carboxypeptidase (hCPZ) is obtained from a normalized cDNA library of pure human DRG (L0001) purchased from Life Technologies, Inc (LTI, Catalog Number: 11315-017, Lot Number: 81027-242). The cDNA inserts are cloned into the EcoR V and Not I sites in 5' to 3' direction into the Gateway-compatible vector, pCMV·SPORT6. Deletion of the frizzled (FZ) domain corresponding to amino acids 42-161, the catalytic (Cat) domain from amino acid 179-568, the point mutant (amino acid E251A), and introducing of a N-terminal FLAG-tag (DYKDDDDK Seq ID. No 1) after amino acid 29 is done by site-directed mutagenesis using primers:
5' CCTCCAGGCCTCCCCGAAGCTTCTCGGCGCTGTCTGCAGCTGGTGGCCTGTGG-3' (Seq Id No. 2) and
5' CCACAGGCCACCAGCTGCAGACAGCGCCGAGAAGCTTCGGGGAGGCCTGGAGG-3' (Seq Id No. 3) for deletion of the frizzled domain,
5' CCACACGGCCAGCCCTCTTCATCCGGGCCAGCCCTGAGGGCAGTGCCTCGTCAGC - 3' (Seq Id No. 4) and
5' GCTGACGAGGCACTGCCCTCAGGGCTGGCCCGGATGAAGAGGGCTGGCCGTGTGG 3' (Seq Id No. 5) for deletion of the catalytic domain,
5' GCATCTCCCGGCCCGCCACCGCGTTGCCATGAATGTTGC-3' (Seq Id No. 6) and
5' GCAACATTCATGGCAACGCGGTGGCGGGCCGGGAGATGC-3' (Seq Id No. 7) for the point mutation E251A, and
5'GGTGGCCTGTGGCATTCACCCTTGTCATCGTCGTCCTTGTAGTCGGCGGGGTTCCGC TCAAACTCG-3' (Seq Id No. 8) and
5'CGAGTTTGAGCGGAACCCCGCCGACTACAAGGACGACGATGACAAGGGTGAATGCCA CAGGCCACC-3' (Seq Id No. 9) for introducing the FLAG-tag. Isolation of all DNA from transformed *E.coli* is performed using Qiagen plasmid kits. The sequence of all open reading frames is verified by DNA sequencing using the ABI Prism 3700 DNA Analyzer system.

In situ hybridizations- Using polymerase chain reaction (PCR) with self-priming oligonucleotide primers flanked in 5' with SP6- and T7-promotor recognition sequences, it is possible to generate riboprobe templates from any known gene sequence without any cloning step. PCR reaction is carried out for 40 cycles with denaturation step at 95°C for 45 sec,

annealing phase at 58°C for 30 sec and extension phase at 70°C for 1 min. After separation on 4 % agarose gel, the PCR products are cleaned with QiAQuick purification kit according to the manufacturer's instructions (Qiagen, Switzerland). The cleaned PCR product is transcribed using T7-RNA polymerase (anti-sense) and SP6-RNA polymerase (sense) at 37°C for 2 hours using dNTP containing Digoxigenin-UTP . Un-incorporated nucleotides are removed, the probe is ethanol-precipitated and dissolved in 50 µl water before storage at – 20°C. Serial dilutions of the DIG-probe and labeled control RNA (known concentration) are spotted onto a nylon membrane. Incubation with alkaline phophatase conjugated anti-DIG antibody is followed by color development phase using NBT/BCIP (75 mg/ml *N*itro *B*lue *T*etrazolium in 70% dimethyl formamide and 30% water and 50 mg/ml 5-*B*romo-4-Chloro-3-*I*ndoyl *P*hosphate in 100% dimethyl formamide), as substrate for the alkaline phosphatase. The CPZ probe concentration is estimated by comparison of the spot intensities with the labeled control RNA. ISH is performed using the fully automated instrument Discovery™ (Ventana Medical Systems, Strasbourg) for in situ hybridization and immunochemistry. The protocol performed to localize this gene using paraffin-embedding tissue section is established within the RNA analytics laboratory and described as below, Deparaffinization and rehydratation of tissues sections is performed on solvent-free conditions using EZprep solution (Ventana Medical Systems SA, Strasbourg) for 8 min at 75°C follwed by 8 more min at 42°C. All pretreatment steps are done with the RiboMap™ kit (Ventana Medical Systems SA, Strasbourg) following the manufacturer's instructions with one additional permeabilization step using enzymatic digestion : optimal results are obtained with 12 µg/ml of proteinase K at 37°C for 16 min. CPZ probe hybridization is performed at 45°C for 6 hours with adapted quantities of DIG-riboprobe (CPZ probe = 5 ng/slide) diluted in RiboHybe solution (Ventana Medical Systems SA, Strasbourg). Post-hybridization washes are performed at 50°C for 8 min on high stringency conditions (0.1 x SSC) for 3 times. For the detection of DIG-label, a biotin-conjugated mouse anti-digoxin antibody (Jackson Immunoresearch Inc.) is applied for 30 min at 37°C after dilution at 1/2000 in antibody diluent and followed BCIP/NBT chromogenic detection is done using BlueMap™ Kit (Ventana Medical Systems SA, Strasbourg) according to manufacturer's instructions. The substrate incubation time for optimal signal / noise balance is 4 hours. Counter staining using ISH nuclear fast red is performed for 10 min. Sections are mounted in Crystal mount and post-mounted using Permount.

Example 1

Presenilin Processing: The observation that the overexpression of cDNAs increased Aβ levels with the C99 substrate suggests an increase in GACE mediated cleavage of APP. Since CPZ and CyD significantly increased the processing of C99 and increased Aβ42 secretion, they are chosen for further investigation. PS1 N-terminal fragment (NTF) levels aere examined in HEK 293 cells transfected with CPZ or CyD. In cells stably transfected with PS1, both full length PS1 and NTFs are easily detectable. In contrast, in non-transfected HEK cells, the endogenous full length PS1 and the NTF levels are low. In cells overexpressing CPZ or CyD, there is a clear increase in PS1 NTF levels indicating these fragment are being generated at higher rates or the endogenous NTFs are being stabilized over the 48 hr transfection period.

Analysis of CPZ: CPZ is a member of the Carboxypeptidase (CP) gene family, in the CPE subfamily, known to process bioactive neuropeptides (Song and Fricker 1997). CPE related enzymes are generally involved in selective processing reactions, by the selective removal of basic residues from the C-terminus of processing intermediates. To determine whether the catalytic activity, expression, or the subcellular localization of CPZ was required to induce Aβ secretion, three mutant constructs of CPZ are generated. In the first construct, the catalytic domain is completely removed (Δcat) leaving only the signal peptide, frizzled domain, and C-terminus. In the second construct, the frizzled domain (Δfz) is deleted, and in the third construct, a single point mutation (Glu251Ala) is introduced into the catalytic domain that would impair the catalytic activity of CPZ. Each CPZ variant is co-transfected into HEK 293 cells with either the APPwt or C99 substrates. Each of the three mutant constructs have no activity as compared to wild type CPZ or the flag tagged CPZ. The results are similar with both the APPwt and C99 substrates.

A possible explanation for the lack of activity of the CPZ mutants is the lack of expression or mislocalization of the protein in the cell. To address this question, the CPZ mutants are flag tagged and overexpressed in CHO cells. In permeabilized cells, CPZ wild-type has a clear plasma membrane or late secretory vesicle localization. This result is consistent with previous studies examining the intracellular distribution of CPZ (Novikova, Reznik et al. 2000). The subcellular distribution remained unchanged for the CPZ mutants constructs; Δcat, Δfz, and Glu251Ala. These findings suggested that the catalytic activity of CPZ, not its intracellular localization or level of expression is required for the induction of Aβ levels in CHO cells.

Disruption of CPZ catalytic activity abolished Aβ production without affecting expression or subcellular distribution. The overexpression of CPZ could compete with Wnt binding to endogenous frizzled receptors resulting in less β-catenin activation (Tesco, Kim et al. 1998). A decrease in β-catenin activity has been associated with increased Aβ production, thought to be mediated by the ability of PS1 to increase β-catenin association with GSK3β (Kang, Soriano et al. 1999). It is possible that CPZ can induce Aβ production by activating PS1 cleavage via caspase-3, resulting in less PS1/β-catenin complexes (Tesco, Kim et al. 1998). If PS1 is not bound by β-catenin then it can more readily associate with the GACE complex. Since frizzled domains are characterized as protein interaction domains, it is possible that the frizzled domain in CPZ could interact with a component of the γ-secretase complex, i.e.-PS1, or APP itself. These interactions are currently being investigated.

Interestingly, the C-terminus of CPZ contains a putative furin cleavage site thought to release CPZ from the plasma membrane into the extracelluar milieu (Novikova, Reznik et al. 2000). Furin is also involved in the release of the Notch ligand Delta from Notch at the cell surface (Ikeuchi and Sisodia 2003). Although the furin site in CPZ has not been confirmed as a furin substrate, it has been demonstrated that CPZ can be secreted from cells (Novikova, Reznik et al. 2000). This suggested that CPZ could be in the same processing pathways as Notch, but how furin cleavage of CPZ is related to Aβ production is currently unclear (Kim, Wang et al. 1999). However, the observation that CPZ, like CyD overexpression, can drive cells into apoptosis, indicates a common mechanism for the two proteins affecting Aβ production.

Previous findings (Novikova and Fricker 1999) suggest that CPZ is expressed in rat brain leptomeningeal cells but CPZ has not been shown to be expressed in regions of the brain known to be affected by Alzheimer's disease (Novikova and Fricker 1999). To determine where CPZ is expressed, mouse brain slices are analyzed by *in situ* hybridization. In wild type C7BL-6 mice (Jackson Laboratories), a CPZ anti-sense probe exhibited binding as a broad distribution in the brain with the cerebellum and frontal cortex having the highest level of signal. Upon closer examination, CPZ is also found to be expressed in the hippocampus near the CA1 & CA3 regions, most likely in the pyramidal cells. In the cerebellum, CPZ is expressed proximal to the molecular region in the Perkinje and granular cell layers. The distribution of CPZ in the frontal cortex did not seem to be localized to a specific cell type, but instead, evenly expressed across the region. The localization of CPZ in

the mouse hippocampus, cerebellum, and frontal cortex demonstrates its expression in regions of the brain known to be affected by Alzheimer's disease in humans.

Characterization of CyD: To test whether these immunophilin-binding compounds could modulate APP processing, HEK/APPswe stable cells are treated with a range of concentrations of cyclosporin A, Sangliferhin A, FK506 or N-methyl-4-valine-cyclosporin concentrations. For each concentration, viability is measured by an MTS assay according to manufacturers instructions (Promega Cat# G5421) and secreted Aβ40 & Aβ42 is measured using the ELISA assay described herein, The viability IC_{50} for all of the compounds is >40μM. The inhibitory IC_{50} for cyclosporin A, Sangliferhin A is < 3uM for Aβ40 & Aβ42 indicating a strong inhibition of Aβ secretion. The IC_{50} for N-methyl-4-valine-cyclosporin is < 10μM for Aβ40 & Aβ42 secretion, while FK506 had no effect on Aβ40 secretion. In contrast, FK506 treatment causes a dose dependent increase in Aβ42 from 3-20μM.

It has been demonstrated that immunosupressants such as cyclosporin A, can inhibit neurodegeneration and apoptosis in several animal models and inhibit Aβ induced mitochondrial damage in isolated mouse mitochondria (Kim 2002). Since CyD is a target of cyclosporin A and localized to the mitochondria, it could be playing a role in Aβ processing at this site, suggesting that CyD may be important for the development of AD in humans. Although no other isoforms have been tested, CyD might have a unique effect on Aβ processing, possibly because it is the only cyclophilin isoform known to localize to the mitochondria. It is found that CyD overexpression in HEK cells could increase caspase-3 activity and stabilize PS1 NTFs suggesting it could directly modify PS1 activity. Furthermore, compounds that can bind and inhibit CyD activity, strongly inhibit both Aβ secretion and C99 cleavage.

In particular, Sanglifehrin A strongly inhibits Aβ at 3μM and is not toxic to the cells until 20μM. Sanglifehrin A is known to bind and inhibit the PPIase activity of CyD with out affecting the ability of CyD to bind to the adenine nucleotide translocator) (ANT) and does not inhibit calcineurin activity like CsA (Samantha J. Clarke 2002). The steep dose response of Sanglifehrin A might be explained in several ways. Sanglifehrin A may inhibit pore opening only when a significant portion of CyD is bound, enough to disrupt its activity at the MPT (Clarke 2002). Since the ANT exists as a dimer, it is likely CyD has to bind ANT with more than one molecule to induce a conformational change. Since Sanglifehrin A has to bind multiple CyD molecules to inhibit its activity on the MPT complex, this would explain the rapid

inhibition of Aβ secretion at the 3 μM threshold. Once this threshold is reached the MPT complex can be completely inhibited and prevent pore opening. This threshold of MPT inhibition also seems to be an important regulator of Aβ processing and/or secretion. Alternatively, Sanglifehrin A could have multiple targets other than or addition to CyD. Whether CyD is regulating the MPT complex directly through its PPIase activity or by a conformational change in the MPT, is not yet clear. In any case, the data presented here and by others are consistent with CyD and the MPT pore playing a key role in Aβ processing.

Although these compounds could dramatically decrease Aβ secretion and inhibit C99 cleavage, they also inhibited Notch cleavage. This was not surprising considering the data suggested CyD and CPZ overexpression affected GACE activity. This data is the first indication that immunophilin compounds can inhibit GACE activity and defines a new pathway that can influence Aβ and Notch processing in the cell.

Interestingly, FK506 treatment dramatically increases Aβ42 secretion in a dose dependant fashion. FK506 is known to bind immunophilin proteins but not to CyD. The specific increase in Aβ42 suggests that FK506 target proteins might be regulating Aβ42 metabolism or production. Both FK506 and CsA can inhibit calcineurin but they have differential affects on Aβ secretion, thus calcineurin is probably not affecting Aβ levels. This unique affect of FK506 further supports the immunophilin pathway regulates Aβ processing.

Example 2

C99 and Notch cleavage

A HEK 293 stable cell line is generated using a modified C99 or Notch transmembrane sequence with a signal peptide, TGN retention sequence, and a GAL4-NLS-VP16 sequence inserted at Q56/Y57 as described in Maltrese, Wilson, et al. 2001. The cells also stably express a 5xGAL4RE-luciferase reporter construct (RD-2002-01437 and RD-2001-02419). These cells measure GACE activity when the C99 transgene is cleaved which activates the Gal4 luciferase reporter. These HEK stable cells are treated with cyclosporin A, Sangliferhin A, FK506 or N-methyl-4-valine-cyclosporin and the IC_{50} for C99 cleavage are determined. GALVP alone may be as the negative control. The GALVP IC_{50} for cyclosporin A, N-methyl-4-valine-cyclosporin Sangliferhin A, and FK506 is 6.9, 9.9, >20, and >20μM, respectively, indicating a clear window between inhibition of C99 cleavage and cell viability, since these compounds are not toxic until > 40μM. However, cyclosporin A, N-methyl-4-valine-cyclosporin Sangliferhin A all inhibit C99 cleavage at 0.71, 0.87, and 0.85μM, and inhibit

Notch cleavage at 1.7, 2.7 and 3.2μM, respectively. FK506 has no effect on C99 or Notch cleavage. Therefore ligands that can bind CyD strongly inhibit C99 and Notch cleavage suggesting GACE activity is being inhibited.

Example 3

Caspase-3 activation

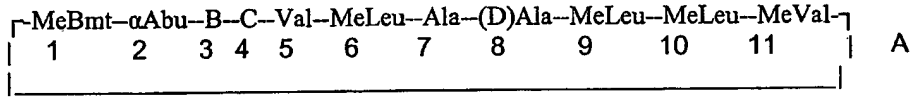
Recent studies suggest that caspase-3 activation can cause an increase in Aβ secretion and stabilize the proteins in the GACE complex (Tesco, Koh et al. 2003). Since CyD and CPZ both increase Aβ secretion and stabilize PS1 NTFs, the levels of caspase-3 activation are analyzed in CyD and CPZ overexpressing cells. HEK 293 cells are transiently transfected with CyD and CPZ along with APPwt for 24 hours, saponin permeabilized, fixed, and probed with a FITC conjugated monoclonal antibody to active caspase-3. Negative control cells are transfected with an empty vector and APPwt and positive control cells are treated with 1uM staurosporine for 6 hours prior to analysis. In cells expressing CyD, 52% of the cells tested positive for active caspase-3, while in CPZ expressing cells, 48% of the cells are positive for caspase-3. These results indicate that the overexpression of these proteins induce the activation of caspase-3 suggesting this is a possible mechanism by which PS1 NTFs are stabilized and Aβ secretion is increased.

CLAIMS

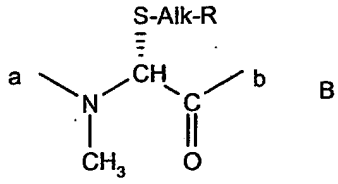
1. Use of a nonimmunosuppresive, cyclophilin-binding cyclosporin in the manufacture of a medicament for treating or preventing pathological conditions associated with Aβ production and/or secretion such as Alzheimer's Disease, Parkinson's Disease, tauopathies, prion diseases, frontotemporal dementia, striatonigral degeneration, Lewy body dementia, Huntington's disease, Pick's disease, amyloidosis, and other neurodegenerative disorders associated with excess Aβ production.

2. A method for the treatment or the prevention of pathological conditions associated with Aβ production and/or secretion, comprising administering to said patient an effective amount of a nonimmunosuppresive, cyclophilin-binding cyclosporin.

3. A use according to claim 1 or a method according to claim 2 in which the nonimmunosuppresive, cyclophilin-binding cyclosporin is a compound of Formula A



wherein B is an amino acid residue of formula B



wherein a denotes the bond to the αAbu residue in position 2;

b denotes the bond to the the residue C in the 4 position;

Alk represents straight or branched chain alkylene containing from 2 to 6 carbon atoms or cycloalkylene containing from 3 to 6 carbon atoms, and

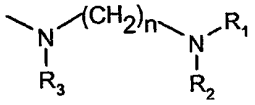
R represents

a carboxy or alkyloxycarbonyl radical;

a radical $-NR_1R_2$ in which R_1 and R_2 are the same or different and represent hydrogen, alkyl, C_{2-4} alkenyl, C_{3-6}cycloalkyl, phenyl (optionally substituted by

halogen, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino) or a benzyl or saturated or unsaturated heterocyclyl radical containing 5 or 6 ring atoms and 1 to 3 heteroatoms, or in which $R_1$and R_2 form together with the nitrogen atom to which they are attached a saturated or unsaturated heterocycle containing 4 to 6 ring atoms and optionally containing a further heteroatom selected from nitrogen, oxygen or sulphur and optionally substituted by alkyl, phenyl or benzyl;

a radical of formula

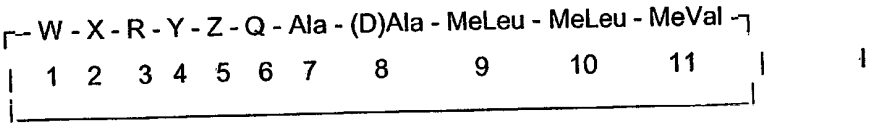


wherein R_1 and R_2 are as defined above, R_3 represents hydrogen or an alkyl radical and n is a whole number from 2 to 4,

and wherein alkyl denotes straight or branched chain alkyl containing from 1 to 4 carbon atoms;

C is MeLeu or or 4-hydroxy-MeLeu; and the pharmaceutically acceptable salts thereof.

4. A use according to claim 1 or a method according to claim 2 in which the nonimmunosuppresive, cyclophilin-binding cyclosporin is a compound of Formula I:



in which W is MeBmt, dihydro-MeBmt or 8'-hydroxy-MeBmt;

X is αAbu, Val, Thr, Nva or O-methyl threonine (MeOThr);

R is Sar or (D)-MeAla;

Y is MeLeu, γ-hydroxy-MeLeu, Melle, MeVal, MeThr, MeAla, Me Tyr, MeTyr(O-$PO(OH)_2$), Mealle or MeaThr, or Pro;

Z is Val, Leu, N-Alk-Val or N-Alk-Leu,

wherein Alk represents Me or Me substituted by

vinyl optionally substituted by

phenyl, or an N S or O heteroaryl containing 6 ring members, or

phenyl optionally substituted by

halogen; and

Q is MeLeu, γ-hydroxy-MeLeu or MeAla.

5. A use according to claim 1 or a method according to claim 2 in which the nonimmunosuppresive, cyclophilin-binding cyclosporin is a compound selected from the group comprising:

a) [dihydro-MeBmt]^1-[γ-hydroxy-MeLeu]^4-Ciclosporin;

b) [MeVal]^4 -Ciclosporin;

c) [MeIle]^4 -Ciclosporin;

d) [MeThr]^4 -Ciclosporin;

e) [γ-hydroxy-MeLeu]^4 -Ciclosporin;

f) [Nva]^2-[γ-hydroxy-MeLeu]^4-Ciclosporin;

g) [γ-hydroxy-MeLeu]^4-[γ-hydroxy-MeLeu]^6 -Ciclosporin;

h) [MeVal]^5-Ciclosporin;

i) [MeOThr]^2-[(D)MeAla]^3-[MeVal]^5-Ciclosporin, or

j) [8'-hydroxy-MeBmt]^1 -Ciclosporin.

m) [N-Benzyl-Val]^5-Ciclosporin,

n) [N-5-Fluoro-Benzyl-Val]^5-Ciclosporin,

o) [N-Allyl-Val]^5-Ciclosporin,

p) [N-3-Phenyl-Allyl-Val]^5-Ciclosporin,

q) [Pro]^4-Ciclosporin, or

r) [γ-hydroxy-MeLeu]^9-Ciclosporin.

6. A use according to claim 1 or a method according to claim 2 in which the nonimmunosuppresive, cyclophilin-binding cyclosporin is [MeVal]^4 -Ciclosporin.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/007556

A. CLASSIFICATION OF SUBJECT MATTER
A61K38/13 C07K7/64 A61P25/00 A61P25/28 A61P25/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 97/18828 A (GUILFORD PHARMACEUTICALS INC; JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDIC) 29 May 1997 (1997-05-29) abstract; claims 39,53,54,57,58; example 4; tables 1,2	1-6
X	WO 03/000281 A (NEURONZ LIMITED; FREYBERG, DEREK, P) 3 January 2003 (2003-01-03) abstract page 9, line 1 - line 6; claims 1,16	1-6

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2005	20/12/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Vandenbogaerde, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/007556

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	SEATON T A ET AL: "Cyclosporin inhibition of apoptosis induced by mitochondrial complex I toxins." BRAIN RESEARCH. 26 OCT 1998, vol. 809, no. 1, 26 October 1998 (1998-10-26), pages 12-17, XP002357773 ISSN: 0006-8993 abstract	1-6
X,P	HANSSON M J ET AL: "The nonimmunosuppressive cyclosporin analogs NIM811 and UNIL025 display nanomolar potencies on permeability transition in brain-derived mitochondria" JOURNAL OF BIOENERGETICS AND BIOMEMBRANES, PLENUM PUBLISHING, NEW YORK, NY, US, vol. 36, no. 4, August 2004 (2004-08), pages 407-413, XP002330699 ISSN: 0145-479X abstract page 412, column 2, paragraph 2	1-5
A	EP 0 484 281 A (SANDOZ LTD; SANDOZ-PATENT-GMBH; SANDOZ ERFINDUNGEN VERWALTUNGSGESELLSC) 6 May 1992 (1992-05-06) cited in the application claims 1-12	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2005/007556

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9718828	A	29-05-1997	AT	246510 T	15-08-2003
			AU	714482 B2	06-01-2000
			AU	7721096 A	11-06-1997
			BR	9611624 A	01-06-1999
			CA	2238050 A1	29-05-1997
			CN	1207043 A	03-02-1999
			CZ	9801540 A3	16-12-1998
			DE	69629392 D1	11-09-2003
			DE	69629392 T2	24-06-2004
			EP	0880358 A1	02-12-1998
			ES	2206608 T3	16-05-2004
			JP	3089350 B2	18-09-2000
			JP	9143198 A	03-06-1997
			LV	12125 A	20-09-1998
			NO	982264 A	20-07-1998
			PL	327994 A1	04-01-1999
			TR	9800890 T2	21-08-1998
			US	6444643 B1	03-09-2002
WO 03000281	A	03-01-2003	NONE		
EP 0484281	A	06-05-1992	AT	148469 T	15-02-1997
			AU	649277 B2	19-05-1994
			AU	8692391 A	07-05-1992
			CA	2054590 A1	03-05-1992
			CS	9103297 A3	13-05-1992
			CY	2159 A	23-08-2002
			DE	69124459 D1	13-03-1997
			DE	69124459 T2	10-07-1997
			DK	484281 T3	17-02-1997
			ES	2095926 T3	01-03-1997
			FI	915135 A	03-05-1992
			GR	3022592 T3	31-05-1997
			GR	3035323 T3	30-04-2001
			HK	1005741 A1	22-01-1999
			HU	62337 A2	28-04-1993
			IE	913824 A1	22-05-1992
			IL	99912 A	12-09-1996
			JP	2740775 B2	15-04-1998
			JP	5208996 A	20-08-1993
			KR	203556 B1	15-06-1999
			MX	9101869 A1	01-04-1993
			NZ	240421 A	28-04-1993
			PL	292248 A1	27-07-1992
			PT	99410 A	30-09-1992
			SK	278808 B6	04-03-1998